Effect of intermittent exposure to 3% CO_2 on respiration, acid-base balance, and calcium-phosphorus metabolism

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Schaefer, K. E., C. R. Carey, J. H. Dougherty, Jr., C. Morgan, and A. A. Messier. 1979. Effect of intermittent exposure to 3% CO_2 on respiration, acid-base balance, and calcium-phosphorus metabolism. Undersea Biomed. Res. Sub. Supp.: S115–S134.—One subject was exposed for six days to increasing levels of CO_2, rising at a constant rate from 0.03 to 3.0% CO_2 within a 15-h period followed by 9 h of air breathing. To assess acid-base parameters, arterialized capillary blood was taken from a finger twice daily (at 8 a.m. and 11 p.m.) at times corresponding to the beginning and end of the intermittent exposure to CO_2. Venous blood samples were obtained on alternate days at the same times. Urine specimens were collected twice daily. The subject was on a liquid diet. Resting respiratory minute volume ($\dot{V}_E$), oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide excretion ($\dot{V}_{CO_2}$), alveolar carbon dioxide and oxygen tension ($P_{A_{CO_2}}$) and ($P_{A_{O_2}}$) were measured twice daily. $P_{A_{CO_2}}$ and $P_{A_{O_2}}$ were also determined at the end of breath-holding twice daily; CO_2 tolerance tests and lung function tests were also carried out. In contrast to the effects of chronic exposure to 3% CO_2, the CO_2 tolerance tests showed an increased sensitivity (increase of slope) and breath-holding $P_{A_{CO_2}}$ did not change, indicating that acclimatization to CO_2 did not develop. The ventilatory response to CO_2 was not sufficient to prevent CO_2 accumulation in the body; this accumulation was eliminated during the nightly air-breathing periods on the fourth and fifth days, indicated by higher values of $P_{a_{CO_2}}$ and $P_{A_{CO_2}}$. The known renal response to hypercapnia, consisting of an increased excretion of titratable acidity, ammonia, and hydrogen ion excretion, occurred but was interrupted after the first day and was triggered again on the fourth and fifth days when accumulated CO_2 was released from body CO_2 stores. The second renal response was associated with a marked calcium excretion, which suggests that bone CO_2 stores were involved.

CO_2
respiration
breath-holding
renal function
calcium metabolism

Several studies have been carried out on the effects of prolonged exposure to increased carbon dioxide concentrations on respiration and acid-base parameters in man (Schaefer 1949;

Sullivan and Dorman 1955; Chapin, Otis, and Rahn 1956; Schaefer 1961; Schaefer, Hastings, Carey, and Nichols 1963; Schaefer, Nichols, and Carey 1964; Schwartz, Brackett, and Cohen 1965; van Ypersele De Strihou, Brasseur, and DeConincok 1966; Glatte, Motsay, and Welch 1967; Brackett, Wingo, Muren, and Salano 1969; Clark, Sinclair, and Welch 1971). There are, however, no comparable investigations of the effect on man of intermittent exposure to CO_2. This problem is of practical significance for snorkel-type submarines, which are submerged during the day and therefore have a rising CO_2 concentration in the atmosphere and which ventilate with air during snorkel operation at night. These submarine conditions were simulated in the present study. The purpose of this investigation was to determine whether body stores are saturated with CO_2 during repeated exposure to CO_2 concentrations increasing from 0.03% to a final value of 3.00% CO_2 at the end of a 15-h exposure and whether the respiratory acidosis induced by intermittent exposure to CO_2 becomes compensated after 5 days, which would correspond to findings for chronic exposure to 3% CO_2 (Schaefer 1949).

MATERIALS AND METHODS

The experimental design consisted of a systematic study of respiration, acid-base balance, calcium metabolism, and visual performance. This required a great number of measurements during the day. Under these conditions the study was limited to one subject, a healthy 23-year-old medical student, thoroughly experienced in all tests performed.

The study was conducted in a large pressure altitude chamber in which constant temperature and humidity could be maintained. Carbon dioxide was admitted from cylinders outside the chamber at a constant rate, resulting in a linear increase of CO_2 from 0.03 to 3% within a period of 15 h. At the end of the 15-h period (11 p.m.), the chamber was opened and ventilated with a fan. During the subsequent 9 h of air breathing, the subject slept. There were three control days prior to intermittent exposure to CO_2 for six days, followed by three days of recovery on air. The subject's vital data were: age, 23 years; height, 5 ft 7 in.; weight, 147 lbs.

Respiratory studies

Resting respiratory minute volume ($\dot{V}_E$), oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide excretion ($\dot{V}_{CO_2}$), alveolar carbon dioxide and oxygen tensions ($P_{A_{CO_2}}$ and $P_{A_{O_2}}$), were measured twice daily, between 8–9 a.m. and 10:30–11p.m. End-tidal gas samples were collected during a 10-min period with a Rahn sampler and mixed expired air was collected in Douglas bags. Carbon dioxide and O_2 concentrations were analyzed continuously with a Beckman LB-1 infrared CO_2 meter and a Servomax O_2 meter. The obtained values were averaged and are reported as alveolar gas tensions. Mixed expired gas was collected in a Douglas bag for the last six minutes of the 10-min test period. Volume measurements were made with a dry gas meter. Oxygen uptake and CO_2 excretion were calculated by adding values of expired and alveolar samples. The respiratory rate was determined with a Yellow Springs thermistor inserted at the side of the mouthpiece.

Carbon dioxide tolerance tests, consisting of a 10-min inhalation of 5% CO_2 in 21% O_2, were carried out on six occasions twice daily, after the measurement of resting ventilation. $\dot{V}_E/P_{A_{CO_2}}$ values obtained at conditions of rest and breathing 5% CO_2 were plotted and the actual slope measured. Breath-holding time and $P_{A_{CO_2}}$ and $P_{A_{O_2}}$ at the breath-hold breaking point were measured every morning between 8–9 a.m. and every evening between 10 and 11 p.m.

Moreover, the time at which diaphragmatic movements occurred prior to the end of breath-holding time was also measured.

Lung volumes and flow rates were determined by the maximal inspiratory-expiratory velocity-volume technique. A Wedge spirometer (Model 370 by Med-Science Electronics), a Tektronic type 502A dual-beam oscilloscope, and a Tektronic oscilloscope camera Model C-12 with a Polaroid film holder were used.

Vital capacity, tidal volume, inspiratory capacity, expiratory reserve volume, maximal expiratory flow rate (MEFR) and maximal inspiratory flow rate (MIFR) were calculated from the photograph.

The subject was trained before experiments commenced. The effort-dependent characteristics of these tests were explained and the importance of a maximal effort was stressed. The flow-volume loops were run in duplicate. If one of the two loops appeared by quick visual inspection to be much smaller in terms of flow or volume, it was discarded and a third determination made. The larger of the two vital capacity values was used; the inspiratory capacity and expiratory reserve volume data were taken from this same photograph. The larger of the two MEFR and MIFR values was chosen, regardless of which determination it occurred in.

Blood studies

To assess the acid-base parameters, capillary blood samples were taken from the finger twice daily, at 8 a.m. and 11 p.m. The capillary blood was arterialized by heating the finger to approximately 45°C for five minutes. The free-flowing arterialized blood was collected anaerobically in heparinized capillary tubes and sealed with clay. These samples were placed on ice and analyzed within 30 min for pH, P_{CO_2}, and P_{O_2} on the Instrumentation Laboratory ultramicro blood gas analyzer, Model 113. Duplicate determinations were made. The validity of equating pH and blood gas tensions of arterialized capillary blood with those of arterial blood has been repeatedly demonstrated by, among others, Gambino (1959).

Venous blood samples were taken twice daily from the antecubital vein at less frequent intervals (every second day) and also analyzed for pH, P_{CO_2}, and P_{O_2}. Moreover, determinations of serum Na, K, and Cl were carried out. Blood lactate and pyruvate were also measured. Unfortunately, serum samples scheduled for Ca, Mg, and P determinations were affected during transport and could not be used.

Urine studies

The collection periods of urine corresponded to the experimental periods of air breathing (11 p.m.–8 a.m.) and CO_2 breathing (8 a.m.–11 p.m.). Urine specimens were collected twice daily at 8 a.m. and 11 p.m. in bottles containing thymol and a layer of mineral oil. Urine volume, pH, CO_2, Ca, Mg, and phosphate were determined, in addition to organic acids, ammonia, and titratable acidity. Net hydrogen ion excretion was calculated (ammonia - titratable acidity - HCO_3). Urinary hydroxysteroids were determined with a modified Porter-Silber procedure (Bray's Clinical Laboratory Methods 1962). Urinary calcium, magnesium, and phosphorus, hydroxyproline and sulfur as well as feces content of calcium, phosphorus, and magnesium were determined in the laboratory of Dr. Bernstein at the Harvard School of Public Health. Throughout the experiment, the subject was on a liquid diet, with a constant liquid intake of 2.25 liters/day and 2795 calories/day. The diet contained 281 mg calcium and 1000 mg phosphorus per day throughout the entire experimental period. An additional 423 mg of calcium

was given in the form of calcium gluconate. Chromium sesquioxide was used as fecal marker. Sodium and potassium were analyzed by an internal standard (lithium) flame photometer. Calcium and magnesium were determined by atomic absorption. Phosphorus was determined according to the method of Fiske and Subbarow (1925).

RESULTS

Respiration

The effect of intermittent exposure to CO_2 on pulmonary ventilation and alveolar gas tensions is shown in Fig. 1 and Table 1. Respiratory minute volume increased to twice the resting ventilation on air at the end of the 15-h exposure to rising CO_2 concentrations reaching 3% CO_2 at the time the measurement was made. Alveolar CO_2 tension rose from 40.7 mmHg (average of 3 daily measurements at 11 p.m.) to 42.4 mmHg (average of 6 daily measurements at 11 p.m. after a 15-h CO_2 exposure). The alveolar O_2 tension increased from 100.7 mmHg to 114.8 mmHg. All the values obtained after 9 h of air breathing remained at control levels, with the exception of the alveolar CO_2 tension, which rose on the fourth and fifth days, reaching a peak

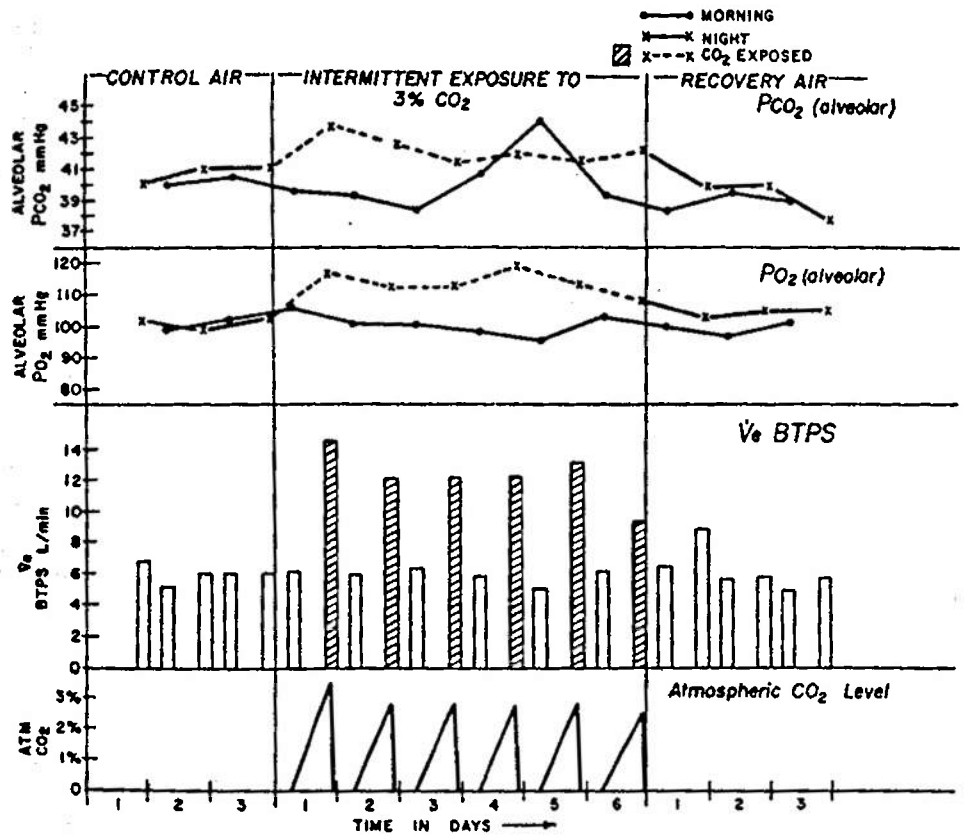

Fig. 1. Effect of intermittent exposure to 3% CO_2 on alveolar P_{CO_2} and P_{O_2} and respiratory minute volume (BTPS). Daily rise of ambient CO_2 from zero to 3% CO_2 over a 15-h period is indicated in lower panel.

TABLE 1

EFFECTS OF INTERMITTENT EXPOSURE TO 3% CO_2

Condition		$\dot{V}_{E(BTPS)}$, liter/min	$P_{A_{CO_2}}$, mmHg	$P_{A_{O_2}}$, mmHg	$\dot{V}_{CO_2}$, ml/min	$\dot{V}_{O_2}$, ml/min	R
Control, 8 a.m.	Mean	5.68	40.7	101.0	186.5	236.0	0.79
	n	2	2	2	2	2	2
Control, 11 p.m.	Mean	6.22	40.7	100.7	205.7	252.7	0.81
	SEM	0.27	1.2	1.2	6.2	8.2	0.01
	n	3	3	3	3	3	3
Experimental: 9 h on air at 8 a.m.	Mean	5.99	40.4	101.8	189.2	233.8	0.86
	SEM	0.17	0.9	1.4	3.0	10.9	0.04
	n	6	6	6	6	6	6
Exposure: 15 h on CO_2 (3% CO_2) 11 p.m.	Mean	12.35**	42.4	114.8**	228.2**	248.5	0.92*
	SEM	0.70	0.4	1.5	7.8	11.1	0.02
	n	6	6	6	6	6	6
Recovery on air, 8 a.m.	Mean	5.79	39.0	100.8	183.3	236.3	0.78
	SEM	0.47	0.2	1.2	12.0	12.2	0.02
	n	3	3	3	3	3	3
Recovery on air, 11 p.m.	Mean	6.59	38.8	105.0			
	SEM	0.70	1.4	0.7			
	n	3	3	3			

*Statistically different from control levels; **significant difference between data obtained after 9 h on air (8 a.m.) and 15 h on CO_2 at 11 p.m. at the 5% level or better.

even higher than the corresponding value on CO_2 breathing. This indicates that the 9-h period of air breathing was not sufficient after three days to eliminate the previously accumulated CO_2.

Data on oxygen consumption, CO_2 excretion, and respiratory exchange ratio are also presented in Table 1 and daily changes in these measures in Fig. 3. Carbon dioxide excretion was consistently elevated during CO_2 breathing, except on Day 6. The oxygen consumption during air breathing declined from 250 to 180 ml on Day 5, a condition which led to an increase of the respiratory exchange ratio during the fourth and fifth days that corresponded to the rise in alveolar CO_2 tension. The subsequent return of the respiratory exchange ratio to near normal values on the sixth day of intermittent CO_2 exposure was paralleled by the fall in alveolar CO_2 tension and a return of both $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ to control values. This suggests that the previously accumulated CO_2 was released from the body CO_2 stores during the two air-breathing periods that comprised a total of 18 h.

The average ventilatory response to inhalation of 5% CO_2 increased during the six-day period of intermittent exposure to 3% CO_2, as shown in Table 2. $P_{A_{CO_2}}$ tended to decrease and $P_{A_{O_2}}$ to rise. The slope of CO_2 tolerance curves increased during the intermittent exposure to CO_2 and during the recovery period on air.

The alveolar P_{CO_2} and P_{O_2} values obtained at the end of breath-holding, together with breath-holding times, are depicted in Fig. 2. The $P_{A_{CO_2}}$ values measured at the end of 15 h of rising ambient CO_2 did not rise above control values obtained under air at 10–11 p.m., indicating that acclimatization to CO_2 did not occur. $P_{A_{CO_2}}$ measured at the end of breath-

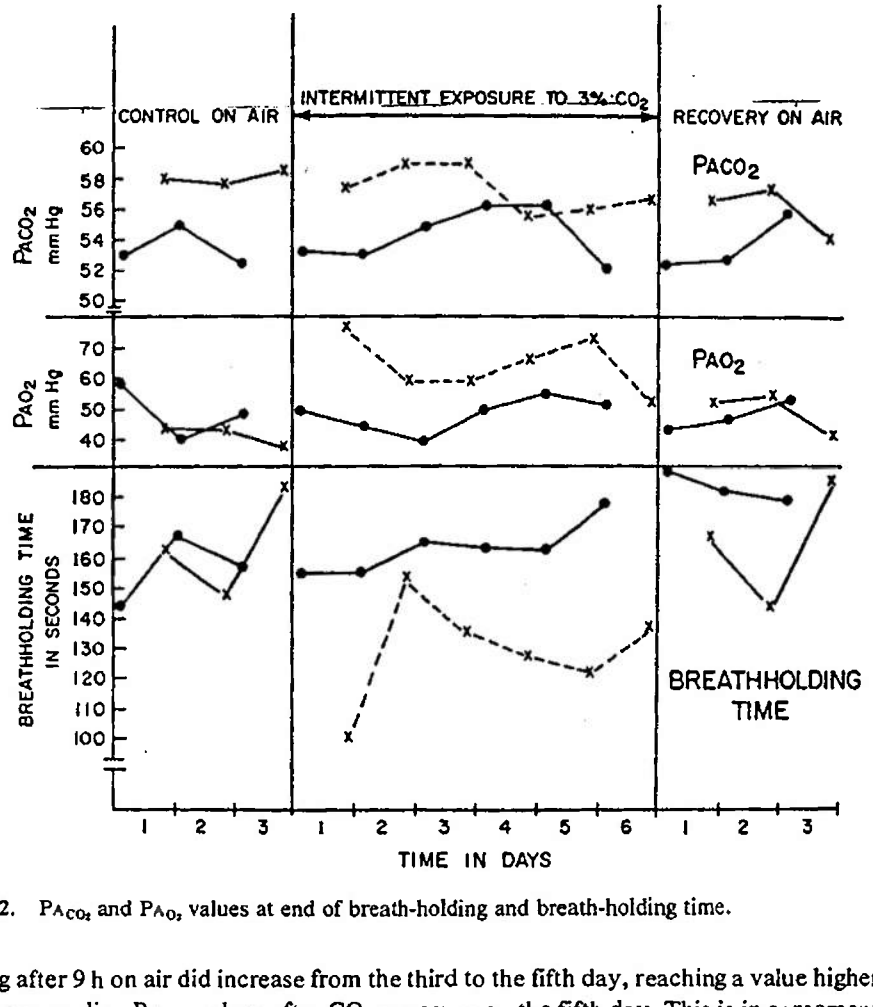

Fig. 2. $P_{A_{CO_2}}$ and $P_{A_{O_2}}$ values at end of breath-holding and breath-holding time.

holding after 9 h on air did increase from the third to the fifth day, reaching a value higher than the corresponding $P_{A_{CO_2}}$ values after CO_2 exposure on the fifth day. This is in agreement with the findings obtained during resting ventilation (Fig. 1).

A summary of the data on breath-holding is presented in Table 3. Breath-holding time and latent time of onset of diaphragmatic movements significantly decreased during intermittent exposure to CO_2. As a consequence, $P_{A_{O_2}}$ increased; $P_{A_{CO_2}}$ remained unchanged.

Intermittent exposure to 3% CO_2 had effects on lung functions (Table 4). Maximum voluntary ventilation (MVV) showed a consistent trend to rise during the experiment. Vital capacity tended to decrease at the end of the 15-h period of CO_2 exposure and also in the morning after a 9-h period of air breathing. The same was true for the expiratory reserve volume, while inspiratory capacity was unaffected during the exposure period. The maximum expiratory flow rates (MEFR) did decrease during the experimental period and they remained below control levels during the recovery period on air, in contrast to the maximal inspiratory flow rates (MIFR), which did not change.

Acid-base status

The acid-base status of the blood (arterialized capillary blood) is exhibited in Fig. 4 and Table 5. Both hydrogen ion concentrations and arterial CO_2 tension showed a small but

TABLE 2
EFFECTS OF INTERMITTENT EXPOSURE TO 5% CO_2 FOR SIX DAYS

Conditions		$\dot{V}_E$ (BTPS), liter/m	$P_{A_{CO_2}}$, mmHg	$P_{A_{O_2}}$, mmHg	Slope of CO_2 tolerance curves
Control	Mean	19.58	49.5	132.7	0.365
	Range	(18.9–20.3)	(49.0–50.0)	(131.7–133.7)	(0.31–0.42)
	n	2	2	2	2
Intermittent CO_2 period of 6 days	Mean	24.37*	47.04	136.7	0.493*
	SE	(1.64)	(0.59)	(0.75)	(0.031)
	n	6	6	6	6
Recovery on air	Mean	21.62	45.06	135.1	0.520*
	SE	(0.80)	(0.33)	(1.77)	(0.042)
	n	4	4	4	4

*Significantly different from controls at the 5% level or better.

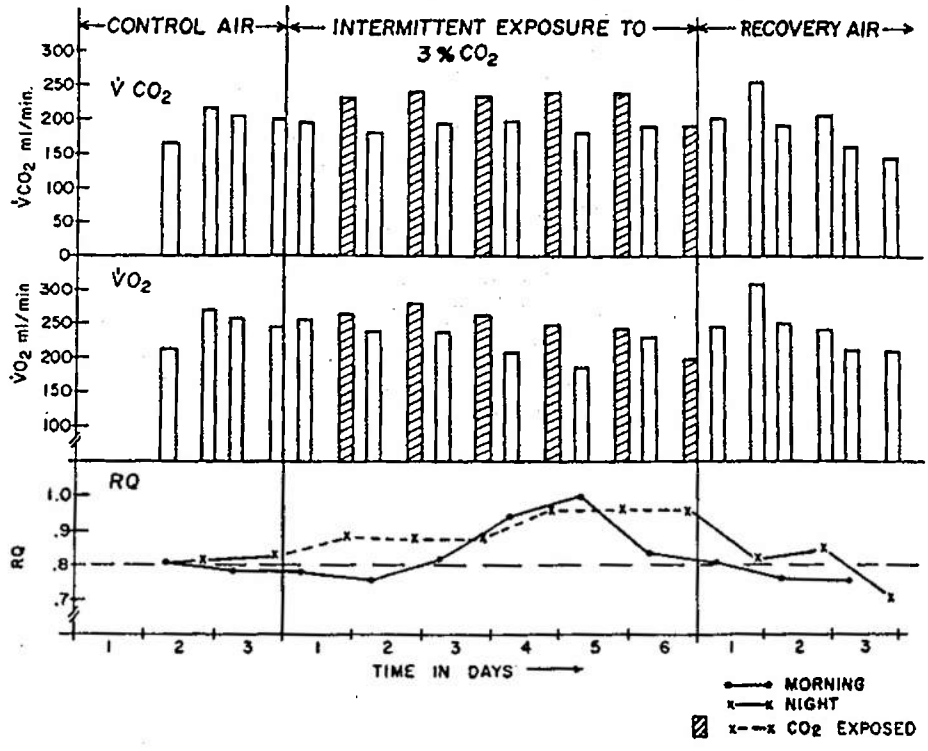

Fig. 3. Effect of intermittent exposure to 3% CO_2 on $\dot{V}_{CO_2}$, $\dot{V}_{O_2}$ and respiratory quotient (RQ).

TABLE 3
EFFECTS OF INTERMITTENT EXPOSURE TO CO_2 FOR SIX DAYS

Condition		Breath-holding Time, s	Diaphragmatic Movements, s	$P_{A_{CO_2}}$, mmHg	$P_{A_{O_2}}$, mmHg
Control, 8 a.m.	Mean	154.8	88	55.3	46.2
	SEM	7.8	4.0	1.9	5.6
	n	3	3	3	3
Control, 11 p.m.		167.7	92.5	58.2	41.8
		3.5	2.5	0.4	1.9
		3	3	3	3
Intermittent Exposure, 9 h on air, 8 a.m.		163.4	88.5	54.3	47.5
		3.5	7.7	0.7	2.1
		6	6	6	6
Intermittent Exposure, 15 h on CO_2, 11 p.m.		141.4*	68.7*	57.3	64.6*
		7.2	5.9	0.6	4.0
		6	6	6	6
Recovery on Air, 8 a.m.		183.5*	110.3*	53.7	47.1
		3.7	2.7	1.1	2.3
		3	3	3	3
Recovery on Air, 11 p.m.		164.7	101.0	56.0	48.6
		12.2	7.5	1.0	4.2
		3	3	3	

*Significantly different from controls at the 5% level or better.

consistent elevation during CO_2 breathing, amounting to 1–2 nM and 1–2 mmHg CO_2.

The values obtained during air breathing remained at control levels, with the exception of the fourth and fifth days, when they rose to higher values than those measured during CO_2 breathing. The blood bicarbonate values did not change significantly under both air breathing and CO_2 exposure. Data obtained from venous blood (Fig. 5 and Table 5) showed similar changes in hydrogen ion concentrations and CO_2 tension during CO_2 exposure. Oxygen tensions in both arterial and venous blood increased during CO_2 breathing (Fig. 6), which confirms the findings on alveolar oxygen tensions.

Blood lactate and pyruvate and L/P ratio were not affected by intermittent exposure to CO_2. The 17-hydroxysteroid excretion in the urine exhibited large diurnal variations that were not influenced by intermittent exposure to CO_2.

To delineate the effects of CO_2 breathing (8 a.m. to 11 p.m.) from air breathing (11 p.m. to 8 a.m.), data on urinary excretion are presented for the corresponding experimental periods; data on 24-h urine excretion would have obscured the effects of CO_2 breathing.

The responses of the renal functions primarily involved in acid-base regulations are shown in Fig. 7. There was an immediate response to CO_2 breathing on the first day, shown by an increase in urine volume and excretion of organic acids, titratable acidity, ammonia, and net acid excretion, followed by a decline in these parameters during the next two days. However, during the fourth and fifth days, which were marked by an increased CO_2 excretion and acid load during the air-breathing period, there was a marked rise in urine volume, organic acids,

TABLE 4
EFFECTS OF INTERMITTENT EXPOSURE TO 3% CO_2 ON LUNG FUNCTIONS

Condition		VC, liters	IC, liters	ERV, liters	MEFR, liter/s	MIFR, liter/s	MVV, liter/min
Control, 8 a.m.	Mean	5.83	3.61	2.22	14.05	12.66	198
	SEM	0.08	0.01	0.08	0.15	0.28	16
	n	3	3	3	3	3	3
Control, 11 p.m.	Mean	5.87	3.95	1.93	13.51	12.18	210
	SEM	0.07	0.10	0.03	0.34	0.54	12
	n	3	3	3	3	3	3
Experimental on air, 8 a.m.	Mean	5.62	3.68	1.94*	12.90*	12.74	225
	SEM	0.05	0.04	0.04	0.27	0.15	3
	n	6	6	6	6	6	6
Experimental on 3% CO_2, 11 p.m.	Mean	5.73	3.97	1.77	12.94*	12.82	225
	SEM	0.03	0.07	0.07	0.16	0.20	5
	n	6	6	6	6	6	6
Recovery, 8 a.m.	Mean	5.67	3.91*	1.76*	12.56*	12.55	244
	SEM	0.06	0.07	0.01	0.11	0.27	3
	n	3	3	3	3	3	3
Recovery, 11 p.m.	Mean	5.80	3.97	1.82	12.45*	12.96	245*
	SEM	0.01	0.14	0.01	0.48	0.23	2
	n	3	3	3	3	3	3

*Significant difference from control levels at the 5% confidence level.

titratable acidity, ammonia, net acid, and hydrogen ion excretion. During the second day of recovery, an opposite trend could be noted, which was characterized by a decreased excretion of ammonia and titratable acidity and a reduction in hydrogen ion excretion commensurate with a large increase in bicarbonate elimination.

Figure 8 exhibits the urinary excretion of chloride, sodium, potassium and bicarbonate. These parameters showed a response pattern similar to that of ammonia and acid excretion: an initial increase on the first day of exposure, a subsequent decline during the second and third days, followed by a marked increase during the fourth and fifth days. On the sixth day of exposure there was again a fall to the level of excretion present on the second and third days. During the first and third days of the recovery period a pronounced increase in urinary excretion of chloride, sodium, and potassium occurred, in contrast to bicarbonate excretion, which reached a peak during the second day of recovery on air.

Urinary excretion of calcium, magnesium, phosphate, hydroxyproline, and sulfur is shown in Fig. 9. Calcium excretion did not show an immediate response the first day of CO_2 exposure. However, it increased between the second to fifth days, coinciding with the filling of CO_2 stores during the second and third days and their subsequent emptying on the fourth and fifth days. During the sixth day of exposure and during the recovery period on air, urine calcium excretion returned to control values. Magnesium excretion fell immediately during the first 15 h of exposure to CO_2, and increased during the subsequent 9 h of air breathing during the night. The second day there was a greatly reduced magnesium excretion both during the CO_2- and air-breathing periods. During the subsequent exposure and recovery periods, magnesium excretion remained below control levels. Phosphate, hydroxyproline, and sulfur excretion in

TABLE 5
EFFECTS OF INTERMITTENT EXPOSURE TO 3% CO_2 ON BLOOD PARAMETERS

Condition		Capillary Blood				Venous Blood			
		pH	P_{CO_2}, mmHg	HCO_3 mM/liter	P_{O_2}, mmHg	pH	P_{CO_2}, mmHg	HCO_3, mM/liter	P_{O_2}, mmHg
Control, 8 a.m.	Mean	7.396	37.2	22.2	86.4	7.360	53.4	29.7	26.6
	SEM	0.004	1.4	0.8	2.2	—	—	—	—
	n	3	3	3	3	1	1	1	1
Control, 11 p.m.	Mean	7.392	36.6	21.6	93.6	7.323	58.2	29.3	20.0
	SEM	0.005	1.7	1.2	3.9	—	—	—	—
	n	3	3	3	3	1	1	1	1
Intermittent Exposure: 9 h on air, 8 a.m.	Mean	7.387	39.8	23.1	86.6	7.319	57.3	28.9	22.2
	SEM	0.006	1.0	0.5	1.5	0.006	1.7	0.5	3.8
	n	6	6	6	6	3	3	3	3
Intermittent Exposure: 15 h on CO_2, 11 p.m.	Mean	7.372*	41.2*	22.9	92.8	7.295*	59.4	28.6	31.9
	SEM	0.003	0.2	0.3	2.2	0.003	2.4	1.3	2.4
	n	6	6	6	6	3	3	3	3
Recovery on air, 8 a.m.	Mean	7.416*	38.3	23.2	78.0	7.338	59.5	31.6	21.4
	SEM	0.001	0.9	1.1	4.2	0.018	0.5	1.1	7.7
	n	3	3	3	3	2	2	2	2
Recovery on air, 11 p.m.	Mean	7.411	38.3	23.8	90.8	7.342	60.8	32.5	21.6
	SEM	0.006	1.0	0.3	0.6	0.005	1.8	0.5	4.5
	n	3	3	3	3	2	2	2	2

*Significantly different from corresponding controls at the 5% level or better.

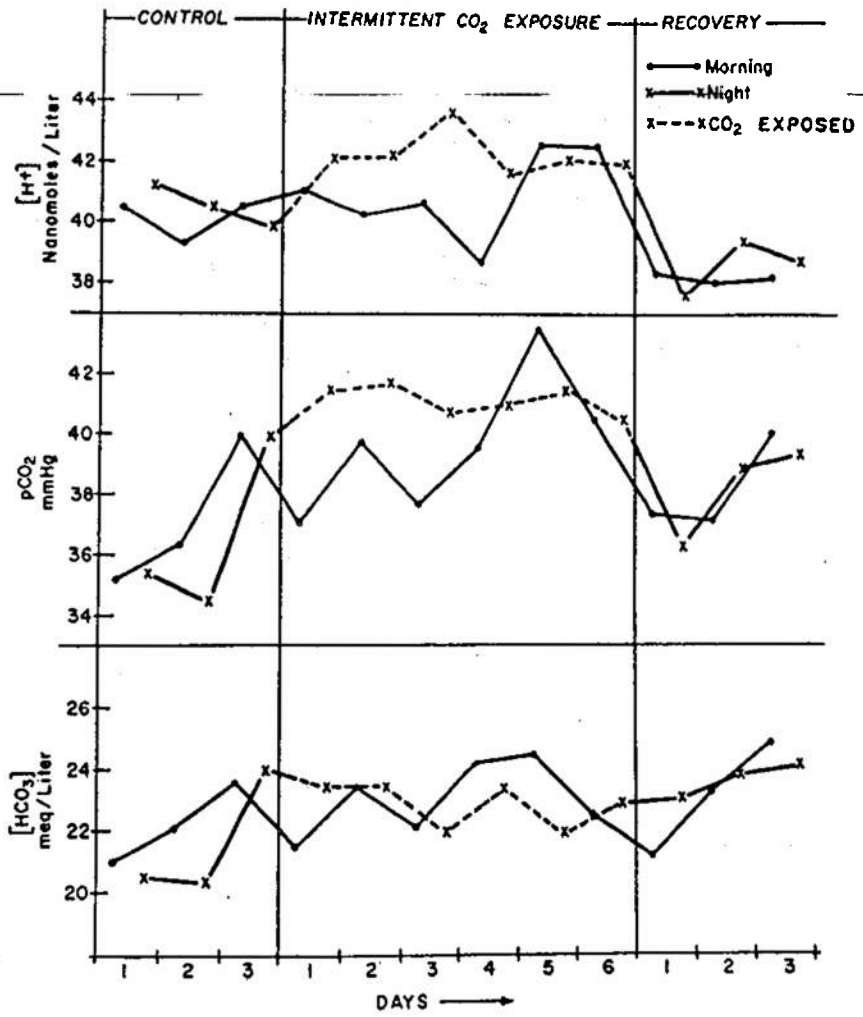

Fig. 4. Effect of intermittent exposure to 3% CO_2 on hydrogen ion concentration, CO_2 tension, and bicarbonate levels of arterialized capillary blood. Solid line, values obtained at 8 a.m. on air; dotted line, values obtained at 11 p.m. at end of CO_2 exposure.

the urine did not change during intermittent exposure to 3% CO_2. Data on calcium, phosphorus, and magnesium excretion are presented in Table 6. During CO_2 exposure, urinary calcium excretion increased; fecal calcium excretion remained practically the same. Phosphate and magnesium excretion in urine and feces decreased during intermittent exposure to 3% CO_2.

DISCUSSION

The experimental design of this study of intermittent exposure to CO_2 required the scheduling of measurements at 8 a.m., prior to eating, and 11 p.m., three hours after supper. Diurnal variations of physiological functions therefore influenced the measurements, and comparisons have to be made of corresponding time periods.

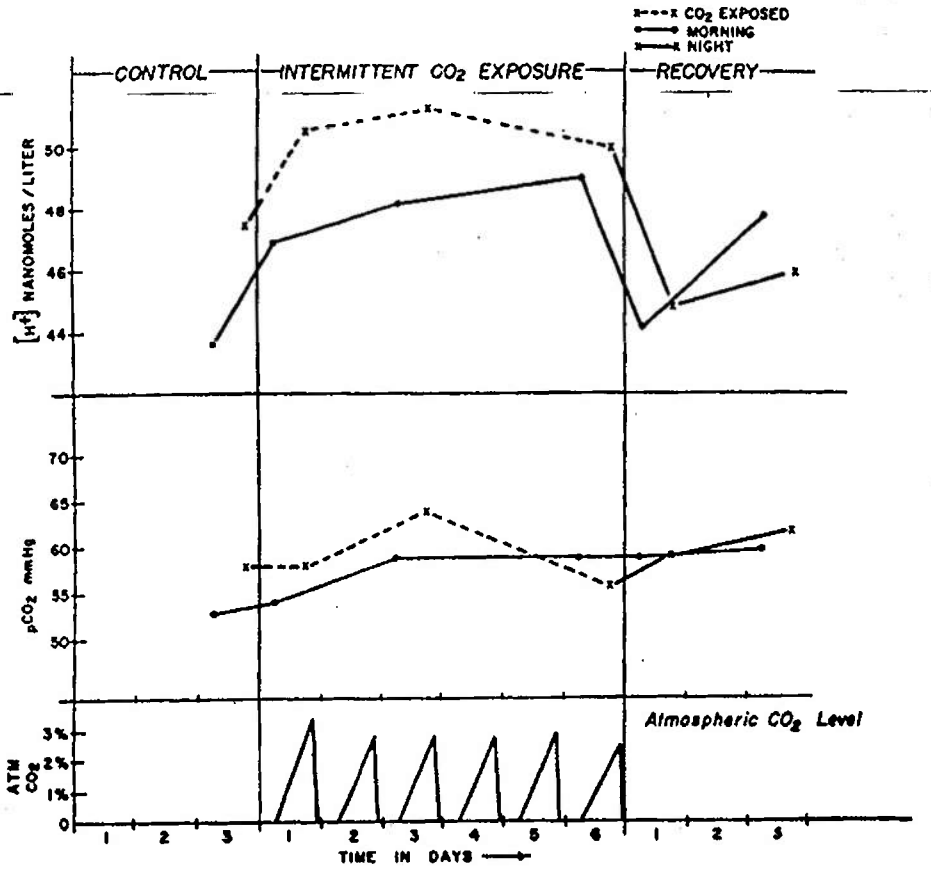

Fig. 5. Effect of intermittent exposure to 3% CO_2 on hydrogen ion concentration, CO_2 tension, and bicarbonate level of venous blood. Solid line, values obtained at 8 a.m. on air; dotted line, values obtained at 11 p.m. at end of CO_2 exposure.

Moreover, the alternating periods of air breathing and CO_2 breathing during intermittent exposure both exhibited CO_2 effects. Carbon dioxide, which accumulated during the previous 15-h period of CO_2 breathing, was not entirely eliminated during the subsequent 9-h period of air breathing. This is indicated by three findings: alveolar P_{CO_2} and arterial P_{CO_2} under resting conditions, as well as $P_{A_{CO_2}}$ at the breath-holding breaking point, rose at the end of the air-breathing period on Day 5 to values higher than corresponding values at the end of the CO_2 breathing period. Enough of the previously accumulated CO_2 during the air-breathing period on Day 5 was eliminated to return alveolar and arterial P_{CO_2} on Day 6 to the levels observed on Days 1 and 2 of the intermittent exposure.

In the recovery period on air, alveolar and blood carbon dioxide quickly returned to control values after 8 h of air breathing and remained at this level, but the main carbon dioxide elimination occurred on the second day of recovery, indicated by a large excretion of urine bicarbonate. This suggested that the metabolic effects of CO_2 were still present at the time of the recovery period when alveolar and blood CO_2 tensions were essentially normal.

At the end of the 15-h exposure to a CO_2 concentration rising from 0–3% CO_2, average respiratory minute volume had increased to twice the control value on air. A marked increase

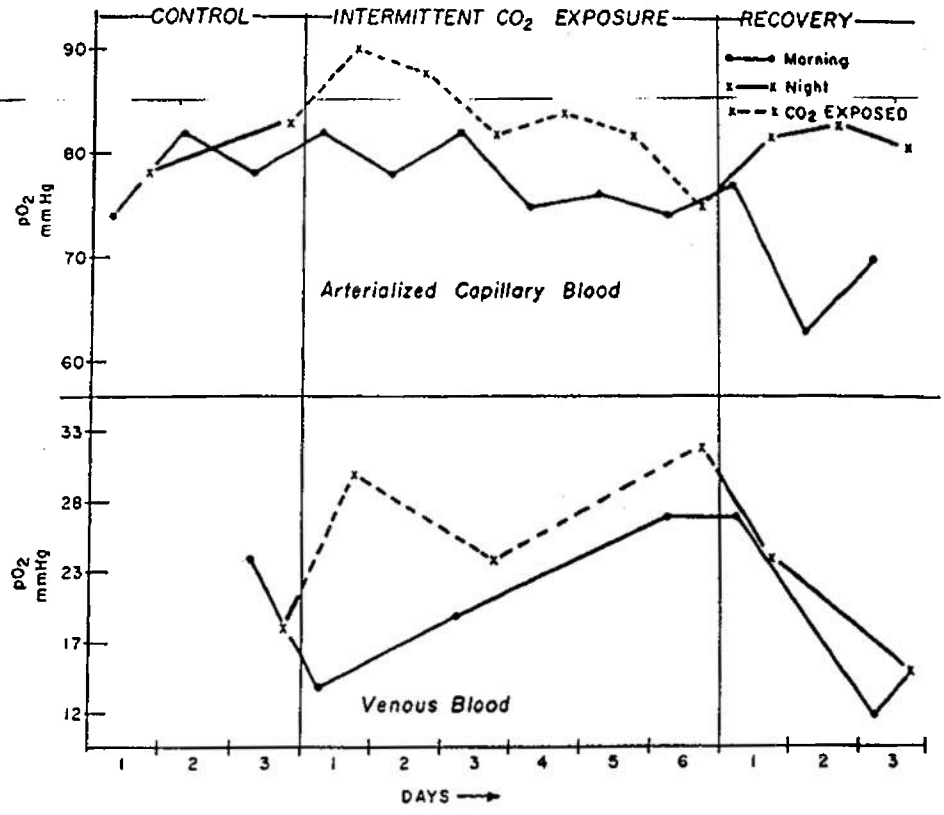

Fig. 6. Effect of intermittent exposure to CO_2 on Po_2 of arterialized capillary blood and venous blood. Solid line, values obtained at 8 a.m. on air; dotted line, values obtained at 11 p.m. at end of CO_2 exposure.

in ventilation tends to minimize changes in alveolar Pco_2 and raise alveolar Po_2. $P_{A_{CO_2}}$ increased by 1.7 mmHg and $P_{A_{O_2}}$ by 14.1 mmHg. These findings are in line with calculations Rahn and Fenn (1955) made for inhalation of 2.8% CO_2 in air: + 3 mmHg $P_{A_{CO_2}}$ and + 16 mmHg $P_{A_{O_2}}$. The average arterial-alveolar difference in Pco_2 ranged between 0.5 and 0.7 mmHg during the air-breathing period of intermittent exposure and during the recovery periods on air. These are reasonable values. However, the arterial-alveolar Pco_2 gradients of 3.5 and 4.1 observed during the control periods were too high, probably because $P_{A_{CO_2}}$ values measured at the beginning of the experiment were too low. During intermittent exposure to CO_2, $\Delta(a-A)Pco_2$ rose from 0.6 to 1.2 mmHg while $\Delta(A-a)\,Po_2$ increased from 15.5 to 22 mmHg. The changes in $(A-a)Po_2$ are similar to those during exposure to 1.5% CO_2, when they increased from 10.6 to 25 mmHg (Schaefer et al. 1963). A significant increase in both arterial-alveolar Pco_2 and Po_2 gradients has been interpreted as indicating an increase in non-perfused and nonventilated alveoli (Schaefer et al. 1963). Trends in this direction were also seen in the present experiment involving intermittent exposure to CO_2.

The effects on respiration of intermittent exposure to 3% CO_2 in this study differ in several ways from the well-known effects of chronic exposure to 3% CO_2. An increased ventilatory response to CO_2 and an increased slope in the CO_2 response curve during intermittent exposure to 3% CO_2 are responses opposite to the depression of ventilatory response and decrease in slope observed in chronic exposure to 3% CO_2 (Schaefer 1949; Clark et al. 1971).

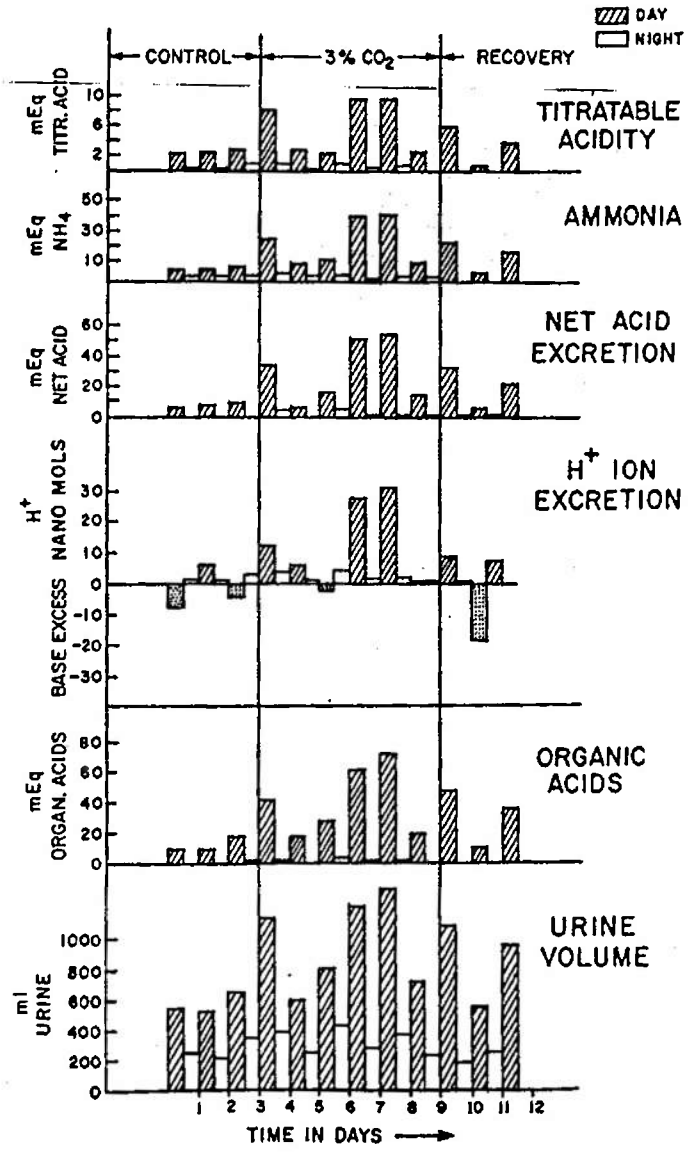

Fig. 7. Effect of intermittent exposure to 3% CO_2 on urine volume and excretion of titratable acidity, ammonia, net acid, H^+ ion excretion, and organic acids in mEq per time period. Black bars, 15-h excretion from 8 a.m.–11 p.m. breathing CO_2; stipled bars, 9-h excretion during night from 11 p.m. to 8 a.m. breathing air.

$P_{A_{CO_2}}$ values at the end of breath-holding did not increase during intermittent exposure to 3% CO_2 (Fig. 2, Table 3), in contrast to the findings obtained by Chapin et al. (1956) in two subjects during chronic exposure to 3% CO_2. After 13 h on 3% CO_2, breath-holding $P_{A_{CO_2}}$ values had reached a stable plateau of approximately 54 mmHg, an increase of 6 mmHg above control values on air. This criterion for CO_2 acclimatization was not attained during intermittent exposure to CO_2.

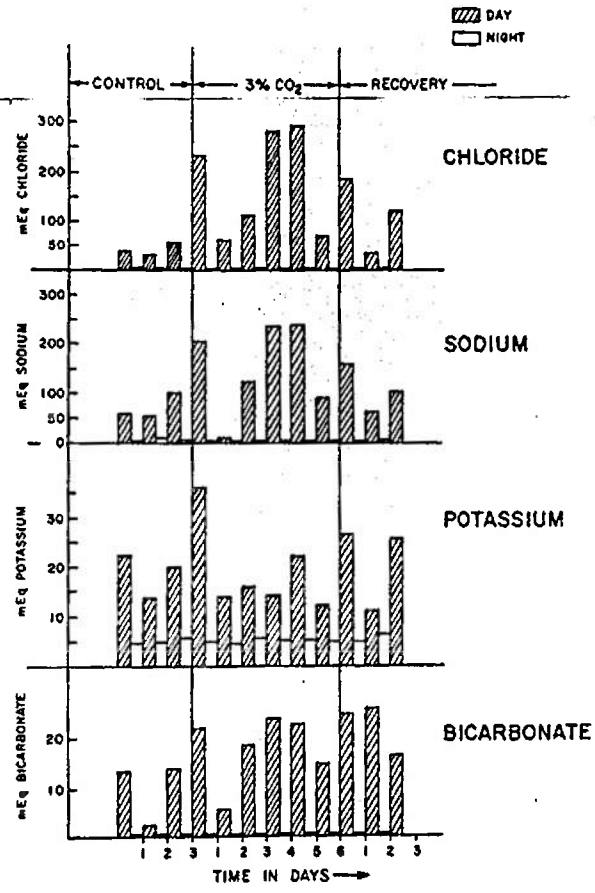

Fig. 8. Effect of intermittent exposure to 3% CO_2 on urinary excretion of chloride, sodium, potassium, and bicarbonate, mEq per time period. Black bars, 15-h excretion from 8 a.m.–11 p.m. breathing CO_2; stipled bars, 9-h excretion during night from 11 p.m. to 8 a.m. breathing air.

The average breath-holding time at the end of the 15-h exposure to rising CO_2 concentration reaching 3% CO_2 was reduced by 27 s, compared to corresponding evening control values at 11 p.m. while breathing air. As a result, the $P_{A_{O_2}}$ values were increased. The fact that $P_{A_{CO_2}}$ did not change during intermittent exposure to CO_2 while breath-holding time was shortened suggests an increased sensitivity to CO_2, which corresponds with the finding of an increased slope in the CO_2 response curve.

A transient increase and decrease in body CO_2 stores within a 5-day period resulted in normal values for alveolar CO_2 tensions and pulmonary gas exchange on the sixth day of intermittent exposure. It is therefore not possible to speak of compensation of a respiratory acidosis.

One similarity between intermittent and chronic exposure to 3% CO_2 was interesting: the same period of five days was required to return to control levels in the first case and to develop a compensation of the respiratory acidosis in the second case.

The steady rise in MVV is probably a training effect. The small increase in IC and corresponding decrease in ERV during the air-breathing periods of intermittent exposure to CO_2 and during the recovery period after CO_2 exposure appears to reflect a fall in FRC. In view of

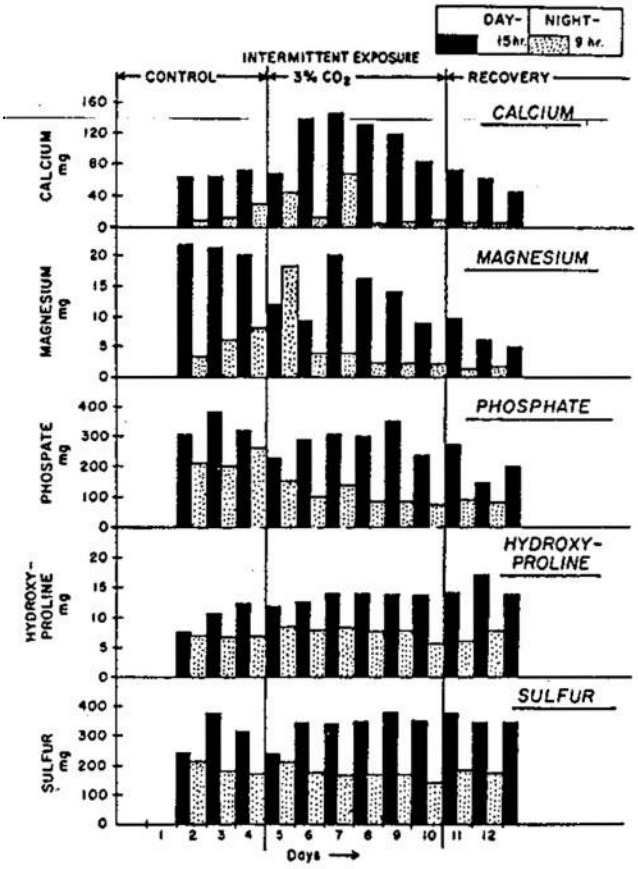

Fig. 9. Effect of intermittent exposure to 3% CO_2 on urinary excretion of calcium, magnesium, phosphate, hydroxyproline, and sulfur, in mEq per time period. Black bars, 15-h excretion from 8 a.m.–11 p.m. breathing CO_2; stipled bars, 9-h excretion during night from 11 p.m. to 8 a.m. breathing air.

these findings it is difficult to interpret the decrease in MEFR of approximately 1 liter/s during both experimental periods and during the recovery period as an increase in airway resistance.

The results of blood gas and pH measurements are in full agreement with the data on alveolar carbon dioxide tensions that show an increase in hydrogen ion concentration and P_{CO_2} in capillary blood during the air-breathing periods of the fourth and fifth days, which indicate a CO_2 accumulation in the organism. The two defense mechanisms against accumulation of CO_2, increased lung ventilation and renal regulation, were apparently not operating efficiently enough to prevent this accumulation. There was an immediate renal response to CO_2 exposure during the first day that consisted of increased excretion of organic acids, titratable acidity, and ammonia, and which returned to control levels during the subsequent second and third days of 15 h of exposure to a rising ambient CO_2 level. This means that the renal response to the CO_2 -induced acidosis was not maintained, as is the case with chronic CO_2 exposure to higher CO_2 concentrations (Schwartz et al. 1965). Based on the available data, it is not possible to explain why the renal response was turned off on Day 2. At the fourth and fifth days, however, when the CO_2 stores of the body began to empty (causing a higher alveolar and blood CO_2 tension at the end of a 9-h air-breathing period), a second and much more pronounced renal response occurred, shown by the large increase in excretion of titrat-

TABLE 6

CALCIUM, PHOSPHORUS, AND MAGNESIUM EXCRETION DURING INTERMITTENT EXPOSURE TO 3% CO_2

			Calcium, mg/day			Phosphorus, mg/day			Magnesium, mg/day		
Control Period	Days		Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
	1		76	353	429	518	554	1062	25	58	83
	2		79	353	432	587	554	1141	28	58	86
	3		105	353	458	591	554	1145	29	58	87
		Mean	86.9	353	439	565	554	1116	27.3	58	85.3
		SE	9.21	0	9.20	23.69	0	27.02	1.20	0	1.2
Intermittent Exposure to 3% CO_2	1		111	365	486	379	442	821	30	41	71
	2		154	365	519	387	442	829	14	41	55
	3		221	365	586	444	442	886	24	41	65
	4		135	365	500	382	442	824	18	41	59
	5		121	365	486	441	442	883	16	41	57
	6		90	365	455	306	442	748	11	41	52
		Mean	138.6*	365	505*	389.8*	442	831.8*	18.8*	41	60*
		SE	18.7		18.3	20.6		20.6	2.86		2.8
Recovery on Air	1		74	551	625	362	598	960	11	60	71
	2		66	551	617	232	598	832	8	60	68
		Mean	70	551	621*	297*	598	896	9.5*	60	69.5*
		SE	4.0		4.0	65.0		64.0	1.5		1.5

*Significantly different from controls at the 5% level or better.

able acidity, organic acids, ammonia, and net acid. At this time hydrogen ion excretion was also markedly elevated. Moreover, calcium excretion appeared to be associated with the phase of renal regulation dealing with the elimination of previously stored CO_2. Urinary calcium excretion was elevated from the second to the fifth days of intermittent exposure to CO_2, in contrast to phosphorus, magnesium, hydroxyproline, and sulfur excretion, which did not show significant changes.

Blood bicarbonate increase is frequently used as a criterion of successful renal bicarbonate reabsorption in response to CO_2 load. Inasmuch as blood bicarbonate did not increase during intermittent exposure to CO_2 (Fig. 4), the renal bicarbonate reabsorption mechanism apparently was not activated, which is in line with findings obtained in animal experiments during prolonged exposure to 1% CO_2 (Schaefer, Pasquale, Messier, and Niemoeller 1979).

The process of CO_2 elimination from CO_2 stores, which was shown by the increased alveolar and blood CO_2 tensions at the end of the nightly air-breathing period, did not involve renal regulation during the night. No change was observed in the pattern of urinary acid excretion during the night. This confirms previous findings of Carey, Schaefer, and Clegg (1966), showing that CO_2 inhalation is not capable of altering the diurnal cycles of acid-base regulation or other diurnal cycles. During the first and third days of recovery on air after six days of intermittent exposure to CO_2, there was another increase in excretion of titratable acidity, organic acids, and ammonia. On the second day of recovery, between the increased levels of acid excretion, there was a strong bicarbonate diuresis, which is in agreement with post-exposure changes seen after prolonged exposure to 3% CO_2 (Glatte et al. 1967) and 1.5% CO_2 (Schaefer et al. 1964).

Although this preliminary study was limited to one subject, it provided some new findings about the limitation of ventilatory and renal responses to CO_2. The major results have been interpreted as follows:

1) The ventilatory response to CO_2 inhalation was not sufficient to prevent CO_2 accumulation in the body.
2) The CO_2 accumulated in the body CO_2 stores was eliminated during the nightly air-breathing periods on the fourth and fifth days, as indicated by the higher blood and alveolar CO_2 values.
3) The renal response to CO_2 inhalation was stimulated during the first day of exposure, but not during the second and third days, and it was again triggered during the fourth and fifth days, when accumulated CO_2 was released from body CO_2 stores.
4) In contrast to the increased CO_2 elimination via the lungs that occurred during the air-breathing period at night, the renal elimination of CO_2 was not carried over into the air-breathing period at night.
5) The second renal response was associated with a marked increase in calcium excretion, which suggests that the bone CO_2 store was involved in the release of CO_2 since bone CO_2 and calcium exchange have been found to be intimately inter-connected (Schaefer et al. 1979).

The subject in this study was on a liquid diet with a known calcium and phosphorus content to ensure that electrolyte excretion, in particular that of calcium and phosphorus, and hydroxyproline excretion would not be influenced by changes in diet. Hydroxyproline excretion did not change from control levels throughout the whole experiment, indicating that bone resorption based on parathyroid stimulation was not involved in the calcium tide associated with the CO_2 release.

It should be mentioned that among the visual tests carried out during this experiment, night vision sensitivity and color sensitivity for green showed impairment repeatedly (Weitzman, Kinney, and Luria 1969).

Although this pilot experiment was limited to one subject, data obtained were of sufficient breadth to warrant a tentative definition of the effects of intermittent exposure to 3% CO_2 on several physiological systems. The observed findings reveal certain limitations of both the respiratory and renal response to CO_2, which were not known to exist before now. New aspects of acid-base regulation through bone buffering during prolonged exposure to 0.8–1% CO_2 on submarine patrols are also reported in this supplement, and these aspects are related to the limitations of the respiratory and renal response to low levels of CO_2 (Schaefer 1979; Messier, Heyder, Braithwaite, McCluggage, Peck, and Schaefer 1979). The results of the experiment with intermittent exposure to 3% CO_2 can therefore serve as a model demonstrating the limitations of respiratory and renal regulation in response to CO_2 exposure.

The subject, *E. K.*, was a medical student enrolled in the Navy Clerkship Program. He had an excellent knowledge of physiological functions, and developed a keen interest in the project. Only through his extraordinary motivation, practical skill in handling equipment, excellent cooperation, and patience was it possible to carry through a study that produced so many demands in the form of simultaneous physiological monitoring and actual performance. The support of Dr. Daniel Bernstein, Harvard School of Public Health, is gratefully acknowledged. Measurements of urinary calcium, magnesium, phosphorus, sulfur, organic acids, and hydroxyproline as well as fecal calcium, phosphorus, and magnesium determinations were carried out in his laboratory.—*Manuscript received for publication March 1978; revision received August 1978.*

Schaefer, K. E., C. R. Carey, J. H. Dougherty, Jr., C. Morgan, and A. A. Messier. 1979. Effets d'expositions intermittentes, à 3% CO_2 sur la respiration, l'equilibre acido-basique, et le métabolisme du calcium et du phosphore. Undersea Biomed. Res. Sub. Suppl.: S115–S134.—Un sujet a été exposé pendant 6 jours à CO_2 dont la concentration est augmentée de 0,03% jusqu'à 3,0% dans une période de quinze heures. L'exposition a été suivie de 9 heures en air. Pour évaluer l'équilibre acido-basique, du sang capillaire "artérialisé" est prélevé au doigt à 8 h et à 23 h (commencement et fin de la période d'exposition). Des échantillons de sang veneux sont prélevés aux mêmes heures d'autres jours. Les échantillons d'urine sont prélevés deux fois par jour. Le sujet reçoit un diète de liquides. Les déterminations de volume respiratoire minute au repos (V_E), la consommation d'oxygène (V_{O_2}), les pressions alvéolaires de CO_2 et de O_2 ($P_{A_{CO_2}}$ et $P_{A_{O_2}}$), et de l'élimination de dioxyde de carbone (V_{CO_2}) sont effectués deux fois par jour. Les pressions alvéolairés sont contrôlées aussi à la fin des exercises d'apnée deux fois par jour. Des tests de tolérance de CO_2 et du fonctionnement pulmonaire sont effectués aussi. Les tests de tolérance a CO_2 traduisent une sensibilité accrue et $P_{A_{CO_2}}$ inchangé; on peut en conclure que l'acclimatation à CO_2 n'a pas eu lieu. La réponse ventilatoire à CO_2 ne suffit pas pour empêcher l'accumulation de CO_2 corporel, accumulation éliminée au cours des périodes de respiration de l'air le 4e et 5e jour, comme le démontrent les valeurs accrues de $P_{A_{CO_2}}$ et de $P_{a_{CO_2}}$. La réponse rénale à l'hypercapnie (élimination plus rapide de l'acidité, de l'ammoniac, et des ions d'hydrogène) est observée; elle disparaît après le premier jour, pour réapparaître le 4e et 5e jours, quand le CO_2 accumulé a été libéré. Cette seconde réponse rénale est associée à une élimination importante de calcium, ce qui fait penser que les dépôts osseux de CO_2 y aient joué un role.

dioxyde de carbone
respiration
apnée
fonction rénale
métabolisme du calcium

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